THE UNIVERSITY OF ALBERTA

THE EFFECT OF PHLEBOTOMY ON SELECTED PHYSIOLOGICAL PARAMETERS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Effect of Phlebotomy on Selected Physiological Parameters", submitted by William Cecil Brown in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The purpose of this thesis was to investigate the effect of a standard blood donation of 500 cc on the physiological parameters of maximal oxygen uptake, maximal oxygen pulse, and maximal heart rate. A subsidiary investigation was conducted on the recovery heart rates of each subject for five minutes following the maximal work load level. These parameters were elicited by a modified version of the maximal oxygen consumption test described by Mitchell, Sproule, and Chapman (42).

Eight healthy male volunteers attending the University of Alberta comprised the experimental group. Each subject was paid a total of eight dollars upon completion of all tests.

For control purposes, a predonation test was administered within one week prior to the blood donation. Subsequent tests were given at intervals of four days, eight days, and twelve days (designated Day 4, Day 8 and Day 12 respectively) after venesection. All blood was drawn by Red Cross personnel during the annual blood donor clinic at the university.

An analysis of variance of the physiological data showed that for maximal oxygen uptake: (1) the Day 4, Day 8 and Day 12 values did not differ significantly from the control level; (2) the Day 4 and Day 8 values did not differ significantly from each other but both were significantly less than the Day 12 value. Similarly, for maximal oxygen pulse, analysis showed: (1) the Day 8 and Day 12 values did not

differ significantly from the control level; (2) the Day 4 value was significantly less than the control level but did not differ significantly from the Day 8 value; (3) the Day 4 and Day 8 values did not differ significantly from each other but both were significantly less than the Day 12 value. Maximal heart rate, however, did not differ significantly between any of the test days. An analysis of variance and trend analyses showed that recovery heart rate was significantly slower for the control test than for any of the other test days which did not differ significantly from each other.

The findings were discussed in terms of possible physiological variables resulting from the blood donation and subsequent recovery.

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CHAPTER I

STATEMENT OF THE PROBLEM

Introduction

Practical experience gained over a large number of blood donations has shown that the loss of about a pint of blood is of no serious consequence to the average healthy adult, provided no immediate strenuous or exacting demands are made upon the donor (6, 20, 39). However, Karpovich and Millman (31) report some difference of opinion as to the desirability of the athlete serving as a donor. Many coaches have reportedly been strongly opposed to the practice of their athletes giving blood during the training season, whereas many athletes have been known to be of the opinion that a blood donation would not seriously affect their performance. Either view may be supported by numerous isolated cases but little scientific evidence has been recorded which deals with the effect of a blood donation on physical performance (6, 12, 27, 31, 56). These studies generally agree that, except for motivational interference performance should decrease after a blood donation. The duration of this decrease when observed has been found to vary. Karpovich and Millman (31) found that performance returned to normal between a few days and three weeks. Balke et al (6) reported the return of performance to the control level two to three days after venesection and a significant increase in performance over the control level eight to ten days after venesection. Although

Howell and Coupe (27) reported no significant change in performance, they did mention trends in performance similar to those observed by Balke _et al_ (6). Their control group, however, showed the same trends. Dennison (12) found a consistent increase in performance over a seven day period in both his psychological control group and his experimental group, but these increases were attributed to motivational learning and/or conditioning. It appears evident that there is no consistent agreement as to the effects of a blood loss on performance. Motivational aspects are present and appear difficult to differentiate from any physiological effects, if they exist.

Another approach which would help to clarify this problem would be to investigate the effect of a blood donation on physiological parameters elicited by a maximal test. The maximal test has the advantage of being independent of motivation and emotion as well as being largely independent of skill (5, 57).

The Problem

This thesis was designed to examine the effect of a blood donation of 500 cc on the physiological parameters of maximal heart rate, maximal oxygen uptake and maximal oxygen pulse at intervals of four days, eight days and twelve days after venesection. These physiological parameters were elicited by a modified version of the maximal treadmill test reported by Mitchell, Sproule and Chapman (42).

According to the null hypothesis it was expected that there would be no significant difference between the control and experimental values of maximal heart rate, maximal oxygen uptake and maximal oxygen pulse.

Subsidiary Problem

It was further proposed to examine the effect of the blood loss upon recovery heart rate. This heart rate was taken at the end of each minute for five minutes following the exercise level at which maximal oxygen uptake is reached.

According to the null hypothesis it was expected that there would be no differences in the rate of heart rate recovery between control and experimental values.

Delimitations

1. The study was limited to eight healthy male subjects registered in the 1967-68 session at the University of Alberta. Because of the nature of the study, these subjects were volunteers, thereby precluding random selection. Also an eight dollar stipend was given to each subject upon completion of all four tests.

Limitations

1. The temperature and humidity of the laboratory could not be precisely controlled therefore it varied from time to time.
2. The amount of blood withdrawn from each subject may have deviated from the normal 500 cc donation through circumstances prevailing at the time.
3. The results are limited by possible errors in testing instruments and machinery although reasonable attempts to control these were made (Appendix C).
4. Because of the non-random selection of subjects, the statistics used were descriptive of this small group or may be

inferred to a hypothetical large population of which these subjects could be considered a random sample.

Definitions

Maximal oxygen uptake. Maximal oxygen uptake is a measure of the ability of the cardiorespiratory system to take up and carry oxygen to the working tissues, and for these tissues to use the oxygen (57). This was taken to have occurred when a measurement of oxygen consumption did not exceed the preceding measurement by 54 ml/min. (42).

Maximal oxygen pulse. The amount of oxygen utilized per heart beat is the oxygen pulse. Maximal oxygen pulse was found by dividing maximal oxygen uptake by the corresponding maximal heart rate (3, 6, 53).

Maximal heart rate. Maximal heart rate occurs at the maximal oxygen uptake value.

Recovery heart rate. After the cessation of exercise heart rate decelerates toward the normal rate. For this study recovery heart rate was the deceleration for five minutes after exercise, taken at the end of each minute.

Healthy subject. One who was to participate in unrestricted physical activity, on the basis of his entrance medical examination, was considered to be a healthy subject.

CHAPTER II

REVIEW OF THE LITERATURE

Influence of Blood Donation on Physical Performance

The earliest recorded interest in the effect of the loss of blood upon physical performance has been reported by Karpovich and Millman in 1942 (31). Their interest arose after a subject who had been training on a bicycle ergometer for nine weeks announced one day (after completing his ride) that he had given 500 cc of blood the previous day. That day his performance did not deviate from the day before. However, the next day his performance dropped sharply and he did not regain his predonation level for about three weeks. Two weeks later, he again donated blood which resulted in an immediate decrease in performance, again lasting about three weeks. Further study by the same investigators on four subjects revealed a decrease in performance after blood donation. The two subjects who were involved in a sprint type of activity quickly regained their proficiency, whereas the two subjects engaged in endurance performance did not regain their previous level for ten to 18 days.

Other isolated cases reported by Karpovich and Millman showed that some athletes improved in performance a short time after giving blood but this was discounted as being "due to a frantic effort to prove that loss of blood did not affect the subjects seriously" (31).

Another study by Spealman, Bixby, Wiley, and Newton in 1948 investigated among other things the influence of controlled hemorrhage

on performance in the heat, using four young male subjects in their early or middle twenties. They found that the removal of 500 cc of blood resulted in immediate and marked decrease in ability to carry out physical activities, consisting of active and passive standing, and exertion on a bicycle ergometer. Several days elapsed before the control level of performance was regained. The authors also found, using themselves as subjects, that performance was affected but to a lesser degree following the removal of 200 cc of blood (56).

Balke, Grillo, Konecci, and Luft (1954) investigated work capacity after venesection and found slightly different results (6). Fourteen staff members between 22 and 45 years of age were divided into two equal groups and their performance was measured on a treadmill (constant speed 3.5 mph, increased slope $\frac{1}{2}^{\circ}$ per minute) before and after a blood donation of 500 cc. Group one was tested one hour, two days, and ten days after venesection. Group two was tested one hour, three days, and eight days after venesection. In both groups a similar pattern of work performance was found. An initial drop in performance occurred after one hour, followed by recovery (to the control level) two to three days later and an increase in performance (above the control level) in eight to ten days. Their results are summarized in Table I.

Dennison (1960) attempted to control for the possibility of psychological factors causing the observed differences in performance after blood loss (13). He divided 20 university athletes into two equal groups on the basis of their test performances. This test consisted of making as many pedal revolutions as possible on a bicycle ergometer with a resistance of 14 kilograms, in a two minute period.

One group gave 500 cc of blood but the other group only thought they gave blood. Subsequent performance was measured two hours, 24 hours, and seven days after the blood donation. Results indicated that both groups improved significantly in performance over all test items; but no statistical difference could be found between the control and experimental groups. These findings were attributable to three possible factors: a psychological desire to prove that the donation had no deleterious effects, learning to operate the bicycle more effectively and, a training effect.

TABLE I

OPTIMAL WORK CAPACITY (Meter-kilograms per minute)

Subjects	Controls	Blood I 1 hour	Blood II 2 days	3 days	Blood III 8 days	10 days
Average Group 1 (7Ss)	970.	903.	940.			1058.
Group 2 (7Ss)	1096.	1002.		1119.	1200.	
Average difference		-93.7	-27.	-8.	+89.3	+76.5
S.D. of the diff.		68.78	93.95	58.8	74.4	57.9
p value		.01	.5	.5	.03	.01

From Balke, Grillo, Konecci, and Luft (6).

Another study reported in 1964 by Howell and Coupe, examined the effect of blood loss on performance in the Balke-Ware treadmill test (27). Twelve first year university students were divided into two equal groups on the basis of their test performance. Both groups

were treated in a similar manner to that reported by Dennison in that both groups thought they had given blood when in fact only the experimental group had given blood. Performance was measured immediately, 24 hours, and seven days after venesection. No significant difference in performance time or between groups was found, however the same trends as observed by Balke et al (6) were reported. These trends may be seen in Figure 1.

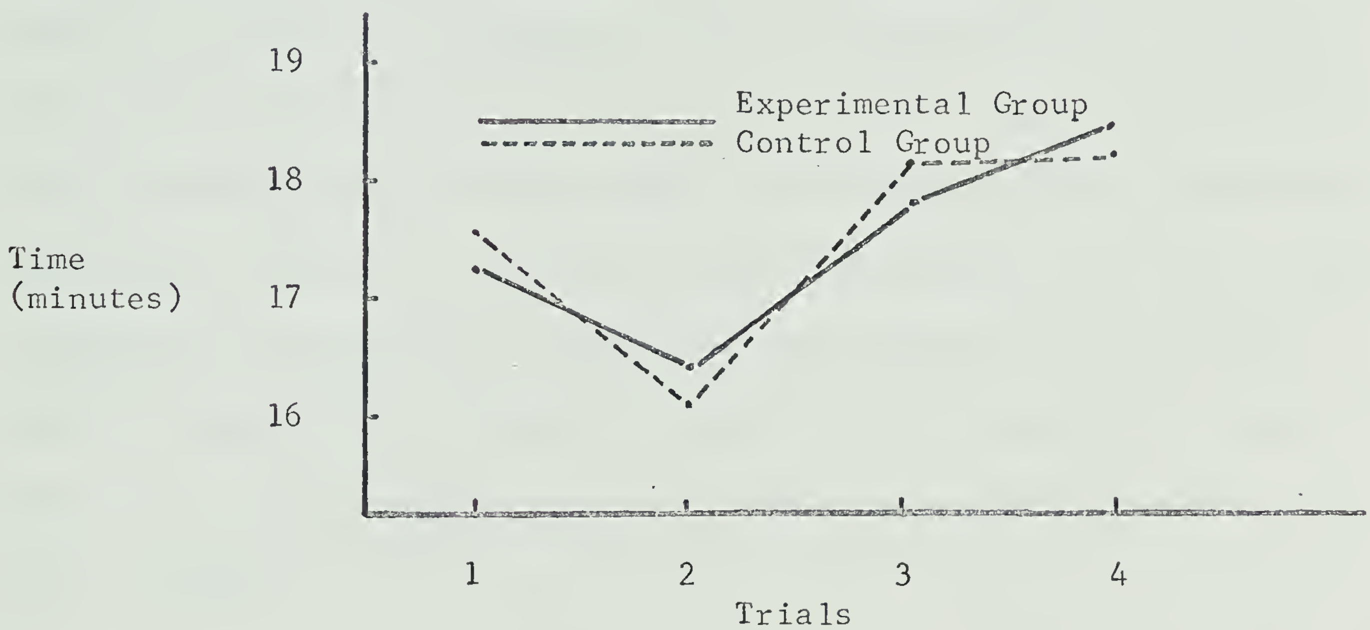

Figure 1: Treadmill performance times for the four trials for experimental and control groups. (From Howell and Coupe (27).)

This study is interesting in that the control group showed the same trends as the experimental group. Motivational aspects obviously have influenced the results.

Influence of Blood Donation on Heart Rate

Hoffman, Litwins, and Sussman (26) have found that if a short rest period (about 10 minutes) is given after the blood donation heart rate will shortly return to normal even after exercise. However Spealman et al (56) have reported an increase in resting pulse rate one half to two hours after the loss of blood. Exercise heart rates

have been reported to increase shortly after a donation by several investigators (6, 26, 56). These increased heart rates vary between about five and thirty beats per minute and are undoubtedly a result of the nature of the exercise. Howell and Coupe (27) found no difference in exercise heart rates after a donation. Although little evidence is available regarding the return of exercise heart rate to normal, it appears to do so after the first day. Balke et al (6) however, have found a bradycardia accompanying their reported increase in performance eight to ten days after the loss of blood. It was conjectured that this observation could be due to overcompensation by plasma for blood lost leading to a greater circulating volume than normal. This explanation is supported by experiments by Spealman et al (56) who found an improvement in physical performance accompanied by a lower heart rate after infusion of serum albumin in a quantity equivalent to 500 cc plasma.

Maximal Oxygen Consumption and Blood Donation

> ... when one subjects a normal individual to progressively increasing workload, allowing sufficient time for recovery between each increment of work, a linear relation between workload and oxygen intake is found. Ultimately, maximal oxygen intake per unit time is reached; beyond this point the workload can usually be increased still further but ordinarily, oxygen intake levels off or declines (42:538).

Maximal oxygen consumption has been frequently used as a measure of physical fitness, and over the years many investigators have endorsed it as the ultimate criterion for physical fitness (22, 57). The use of a test of maximal oxygen consumption has the following advantages:

1. Maximal oxygen consumption values are independent of motivation and emotion and largely independent of skill (8, 57).

2. Maximal oxygen consumption values are stable and repeatable. Taylor, Buskirk, and Henschel (57) reported a test-retest reliability of .95 stable over one year. Other reliabilities have been found to be .926 (10) .968 (42) and .95 (38).

Only two studies are available which have examined oxygen consumption as it is affected by a blood loss. Howell and Coupe (27) reported oxygen consumption values for two minutes before exercises, and for one minute, two to three minutes, and four to five minutes of recovery. The mean pre-exercise oxygen consumption for the experimental group showed a consistent decrease from trial one to trial four (before to seven days after donation). No other consistent pattern was found. The significance of this pattern was not discussed.

Balke et al (6) measured maximal oxygen consumption on their test before venesection, and one hour, two days, three days, eight days, and ten days after venesection. They found a significant decrease (about 9%) in maximal oxygen consumption one hour after blood loss. Two to three days later it returned to the predonation (control) level and eight to ten days later an increase (about 5%) was recorded, Table II.

TABLE II

MAXIMAL OXYGEN INTAKE (ML/MIN)

Subjects	Controls	Blood I 1 hour	Blood II 2 days	3 days	Blood III 8 days	10 days
Average						
Group 1 (7Ss)	2848	2511	2698			2863
Group 2 (7Ss)	3049	2808		3087	3293	
Average difference		-309.6	148.3	+15	+221.4	-3.97
S.D. of the difference		179.2	393.5	180	235.8	290.2
p value		.01	.4	.5	.05	.5

(From Balke, Grillo, Konecci, and Luft (6).)

Oxygen Pulse and Blood Donation

Oxygen pulse has been defined as "the amount of oxygen consumed by the body from the blood of one systolic discharge of the heart" (53:198). It is one of the main factors determining the total amount of energy that can be commanded during exertion (53). The oxygen pulse gives an index of the output of the heart per beat and, according to Schneider (53), this is probably a reflection of the stroke volume. More recent experimental findings, however, indicate that oxygen pulse would more correctly be an indication of both stroke volume and arteriovenous oxygen difference of the blood (64, 42). In the work of Mitchell, Sproule, and Chapman (42) maximal oxygen uptake (MVO_2) was found to be dependent primarily upon heart rate (HR), stroke volume (SV) and arteriovenous oxygen difference (AVO_2 difference).

In equation form this would be:

$$MVO_2 = SV \times AVO_2 \text{ difference} \times HR.$$

Since oxygen uptake divided by the corresponding heart rate is equal to oxygen pulse (O_2 pulse) (53:198; 30:135), the following relationship is evident:

$$O_2 \text{ pulse} = SV \times AVO_2 \text{ difference.}$$

For any given oxygen uptake value, oxygen pulse decreases with increasing heart rate and increases with decreasing heart rate. Oxygen pulse is therefore considered to be a function of stroke volume as well as the difference in oxygen content between arterial and mixed venous blood (3). According to Fick's principle, for a given total oxygen consumption, the arterio-venous oxygen difference is inversely proportional to the minute volume of the heart; hence it can be said that differences in oxygen pulse for the same rate of oxygen reflect changes in the ratio of stroke volume to cardiac minute volume. This relationship has considerable bearing on the economy of cardiac function (3, 6).

Balke et al (6) found that by comparing the oxygen pulse at points of equal oxygen consumption, the stroke volume must have been small relative to the minute volume in the tests immediately after the blood donation. In the tests ran eight to ten days after the blood donation the average oxygen pulse was significantly higher than in any of the other tests, which indicates that total cardiac output was accomplished with a relatively large stroke volume. (This condition appears to support the significantly higher total oxygen intake reported above.)

Recovery Heart Rate and Blood Donation

The time required for pulse rate to return to normal after exercise depends primarily upon the intensity of exercise and upon the condition of the individual (30, 40, 53, 44). Increasing intensity of exercise tends to increase the time required for recovery. On the other hand, better physical condition tends to shorten the period of recovery.

Studies conducted on the pulse rate of subjects after strenuous exercise have shown that post exercise pulse rates follow exponential curves and suitable formulae have been evolved to describe them (30, 28). The regularity and predictability of recovery heart rates after exercise have led to the development of many tests which utilize recovery heart rates to create an index of cardiovascular fitness. Some of these tests are: Fosters test, Gallagher and Brouha Tests for high school boys and girls, Harvard Step Test, and the Tuttle Pulse Ratio Test (40).

Howell and Coupe (27) examined recovery heart rates for five minutes following exercise on the Balke-Ware treadmill test. They reported no observable pattern differences for recovery heart rate attributable to a blood donation.

Response of the Cardiovascular System to Blood Loss

The capacity of the venous system is three to four times greater than the arterial system and can readily adjust to wide variations in blood volume. In response to a loss of blood there is an almost immediate increase in vasomotor tone and vasoconstriction. This tends to reduce venous capacity and restore the ratio between volume and capacity (1). The only possible way by which the circulating

volume can be quickly increased is by expansion of the plasma volume with inflow of protein poor fluid into the vascular system from the interstitial spaces, resulting in hemodilation (1, 12, 14). A short time later large quantities of protein pass by way of the lymphatics into the protein poor plasma to help restore it to normal level. Additional protein as it is needed is formed within a few days in the liver (25).

During the process of hemodilution, hemoglobin concentration decreases until it reaches a minimum value when the hemodilution is complete. Total hemoglobin changes little or none during hemodilution. As the red cell volume increases both hemoglobin concentration and total hemoglobin content increases. When total hemoglobin reaches its pre-donation level, hemoglobin concentration is usually still below normal indicating a greater than normal volume (6, 12, 25, 47, 56). The significance of the greater than normal volume has been discussed previously in regard to heart rate and oxygen pulse.

The red cell volume cannot change rapidly since there are no red cell reservoirs in the body, therefore red cell volume deficiency is compensated for by an increase in the rate of erthropoiesis, which is a relatively slow process (1). The primary purpose of erythrocytes is to transport oxygen from the lungs to the various tissues and to return the excretory gaseous products (primarily carbon dioxide) from the tissues to the lungs. Under normal conditions this requires the passage of a minimal number of erythrocytes through the pulmonary circulation per unit time. When the flow of erythrocytes falls below this critical level, the circulatory rate and the depth and rate of

respiration is increased. If however, these measures fail to account for the deficiency or are required for long periods of time, the rate of erythropoiesis is increased. The stimulus for erythropoiesis is related to the size of the deficit of 'effective' circulating erythrocytes. The greatest stimulus to erythropoiesis, therefore, is a decrease in oxygen carrying capacity, usually by the acute loss of intact blood cells from the circulating blood (53). Erythropoiesis may compensate from four to ten times normal production rate beyond which further demands may go unanswered (25, 53). In certain pathological conditions, however, in which the erythropoietic system is stimulated over long periods of time, the rate of erythropoiesis may be extended even further (50 to 100 times normal) (53).

Experimental evidence has shown that there exists a wide variety of interindividual difference in regenerative ability after a blood loss. Jones, Widing, and Nelson (29) studied 50 donors who had given blood for a total of 175 donations and reported that the hemoglobin level rapidly returned to normal. Some donors exhibited such regenerative ability that after a loss of 1200 to 2000 cc of blood within one to 23 days hemoglobin level returned to normal within ten days. Martin and Myers (39) made observations on ten donors who had given from one to 23 donations each. They reported that the reduction in erythrocytes, when 500 cc of blood was taken, was regained usually in four to six days. In another study, four healthy young men donated 500 cc of blood, and it was found that total hemoglobin regeneration was complete after four weeks, although hemoglobin concentration was still low (47). Fowler and Barer (20) further reported that total hemoglobin returned to normal between 18 and 98 days after donations

of 475 to 600 cc of blood. Another investigator has stated that three to four weeks is necessary for total hemoglobin to return to normal after a 400 cc blood loss (60).

Similar discrepancies may be found in time required for fluid volume to be replaced after a blood loss. Guyton (25) reports that volume is replenished in one to two days. Martin and Myers (39) report a time of six hours after 500 cc of blood loss. DeGowin et al (12) after removing 1000 to 1150 cc of blood reported from five to 96 hours before blood volume was replaced. Seven to fourteen days was required to replace the volume of a 400 cc donation as reported by Wadsworth (60). Ebert et al (14) report that after donations of 1000 cc volume was replaced within 36 hours.

The above reported discrepancies in regeneration times following blood loss are difficult to explain. Normal rate of red cell production is approximately enough to form 1250 ml of new blood per month (or about 42 ml per day) (25). If erythropoiesis increased only two times this normal amount it would take about 12 days for a 500 cc donation to be replaced. Perhaps another factor or factors not evident in the experimental reports have influenced the values obtained.

CHAPTER III

METHODS AND PROCEDURES

Subjects

The experimental group consisted of eight healthy male students attending the 1967-68 session at the University of Alberta, Edmonton. All were paid volunteers, and no attempt was made to select them at random.

Test Design

Each subject was required to donate 500 cc of blood to the Red Cross as well as to complete four maximal oxygen uptake tests. The first test was given within one week prior to the donation. Subsequent tests were given four days, eight days, and 12 days respectively after donating blood. The entire study was centered around the Red Cross blood donation drive at the University between October 30 and November 9, 1967.

The basic test design and subsequent analysis was that of a single factor experiment having repeated measures on the same elements. The major advantage of this statistical model is to provide a control for differences between subjects, by each subject acting as his own control. In this way differences in the performances of the subjects is eliminated from the experimental error assuming that an additive model is appropriate (62).

Organization

Each subject was brought into the laboratory before the first test for orientation. The purpose and procedure of the subsequent donation and testing was carefully explained. Adequate opportunity was given the subjects to become acquainted with the treadmill and respiratory apparatus. At this time arrangement was made for the first test and a sheet of instructions and information regarding possible donation and subsequent test dates were given to each subject (Appendix A).

Upon completion of the first test, the subject committed himself to a donation date and the accompanying dates for the second, third, and fourth tests.

Age, height, weight, and previous experience as a blood donor was recorded after the last test. The subject was also paid at this time.

Standardization of the Testing Situation

It has been found by various investigators that maximal oxygen uptake and heart rate can be affected by: temperature variation (44, 57), ingestion of food (57), previous activity (37), and smoking. Therefore the following standard procedures were followed:

1. Laboratory temperature was maintained at $72 \pm 4^{\circ}$ F.
2. Subjects were requested not to smoke two hours prior to the testing.
3. No test was scheduled, as far as it was possible, sooner than one and one-half hours after a meal. The tests were also scheduled for the same part of the day.

4. The subjects were requested to refrain from any abnormal or strenuous exercise within two hours of the testing.

Respiratory Apparatus

Room air was inhaled through a low-resistance triple-J respiratory valve and expired through the same valve into a 200 liter Douglas bag. The respiratory valve and Douglas bag were connected by a one and one-half inch diameter flexible plastic hose and a Thomas valve. A light-weight headgear was worn by the subject to support the respiratory valve and hose.

Heart Rate Recording

Heart rate was recorded by a Sanborn 100 Viso-Electro-cardiograph. The electrode leads were strapped to the subject in the following manner:

LA lead - over the fifth intercostal space, under the left nipple.

RA lead - over the fifth intercostal space, under the right nipple.

RL lead - over the tip of the right scapula.

Maximal Oxygen Uptake Test

The maximal oxygen uptake test used, was a modified version of the one described by Mitchell, Sproule, and Chapman (42). The test was performed on a motor driven treadmill according to the following procedure:

1. The subject was first hooked up to the electrocardiograph and then instructed to walk at three miles per hour on a ten

per cent grade. This warm-up was followed by a ten minute rest in the sitting position.

2. During the rest period the respiratory apparatus was adjusted to the subject and checked for normal operation.

3. Exercise runs were carried out at a speed of six miles per hour for two minutes and 30 seconds and expired air was collected in a Douglas bag between one minute 30 seconds and two minutes 30 seconds of the run. The first exercise run was carried out at seven and one-half per cent grade.

4. Analysis of the expired air was immediately carried out during the intervening ten minute rest.

5. After the rest period, the work load was increased by raising the grade two and one-half per cent, the speed being held at six miles per hour, and the procedure was repeated until the oxygen intake measured in liters per minute, levelled off or declined. The criterion for deciding whether or not maximal oxygen uptake had been reached, (if it did not decline) has been determined by Mitchell, Sproule, and Chapman (42) to be a difference of less than 0.054 liters of oxygen per minute between two successive tests.

6. When it appeared evident that a subject could not complete a given level, a partial gas sample was taken.

7. Heart rate was monitored immediately after the treadmill stopped at each level. Recovery heart rate was recorded at the end of each minute of rest for five minutes.

An individual score sheet for these values and calculation of MVO_2 was used (Appendix B).

Gas Analysis

The expired air in the Douglas bag was shaken in order to mix the air thoroughly. A sample was then pumped from the bag through a Beckman #E-2 oxygen analyzer via one-quarter inch vinyl tubing. The percentage of carbon dioxide was determined in a similar manner using a #KK Godart Capnograph infra-red carbon dioxide analyzer. The volume of expired air was determined by passing the contents of the Douglas bag through a #802 American Volume Meter. These values were used to calculate oxygen consumption by a standard mathematical procedure as shown in Appendix B.

Calibration of Equipment

Both gas analyzers were carefully calibrated prior to use each day and at regular intervals during testing. Treadmill elevation and volume meter operation were also calibrated. These standard procedures are reported in Appendix C.

Statistical Analysis

1. Maximal values of heart rate, oxygen consumption and oxygen pulse were tested by means of an analysis of variance for a single factor experiment having repeated measures (62:105-132).
2. Duncan's New Multiple Range Test was used to determine significance of differences between means (15:136-140).
3. Trend analyses were done on the three maximal measures over the four testing days (62:70-77).
4. Recovery heart rate was analyzed in terms of a two factor experiment in which both factors (minutes of recovery and days) were repeated measures (62:289).

5. The day by minute interaction term for recovery heart rate was analyzed by means of a trend comparison technique (16:314-316).

Significance was considered at $\alpha = .05$.

CHAPTER IV

RESULTS AND DISCUSSION

Subjects

The means, standard deviations and ranges of height, weight and age for the experimental subjects appear in Table III.

TABLE III

MEANS, STANDARD DEVIATIONS AND RANGES OF HEIGHT, WEIGHT, AND AGE FOR THE EXPERIMENTAL GROUP

	Mean	Standard Deviation	Range
Age (years)	22.2	3.9	18.3-27.8
Height (inches)	70.7	1.4	68.5-72.0
Weight (pounds)	169.9	12.6	154.0-185.0

Maximal Oxygen Uptake

The obtained values for maximal oxygen uptake, with their means and standard deviations before the blood donation, four days, eight days, and twelve days after the blood donation (designated Predonation, Day 4, Day 8, and Day 12 respectively) are presented in Table IV. Values for subjects five and eight (contained in parentheses) have been estimated since they were unavailable. The method of estimation used is described by Goulden (23:318-327). Mean values for the maximal oxygen uptake tests have been plotted in Figure 2.

TABLE IV

MAXIMAL OXYGEN UPTAKE VALUES IN LITERS PER MINUTE

Subject	Predonation	Day 4	Day 8	Day 12
1	4.053	3.845	3.627	3.892
2	3.961	3.476	3.532	3.662
3	3.730	3.689	3.466	4.480
4	3.943	3.946	3.834	4.292
5	3.764	3.335	3.708	(3.855)
6	3.814	3.539	3.289	3.545
7	3.802	3.507	4.217	4.476
8	3.916	3.807	(3.737)	4.000
Mean	3.873	3.643	3.676	4.025
S.D.	.107	.198	.260	.333

Note: Values in parentheses have been estimated according to Goulden (23:318-327).

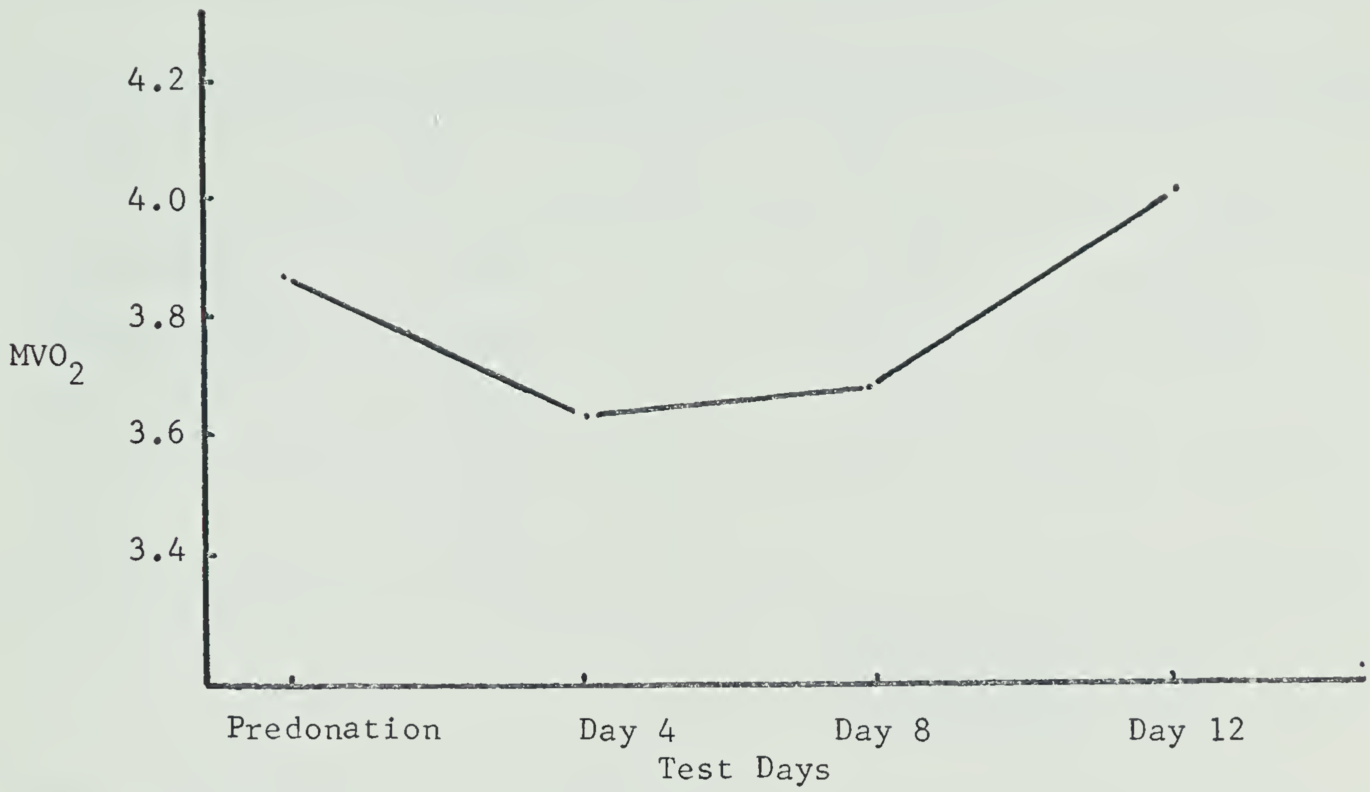

Figure 2: Mean maximal oxygen uptake values (MVO_2) for the experimental subjects before and at the three specified periods after the blood donation.

Homogeneity of variance between test days was tested by the Cochran Test (62:94). The obtained C value (.484) was less than the critical C value (.536 at the .05 level with four and seven degrees of freedom), indicating that the variances were homogeneous, therefore satisfying that assumption inherent in the analysis of variance.

An analysis of variance was performed on the data of Table IV (62:105-132) making appropriate adjustments to the degrees of freedom and sums of squares for the between subject variation and the between test variation (23:323). Table V shows the summary analysis of variance table for the maximal oxygen uptake values. The F ratio (4.18) for the between test variability was significant at the .05 level. Intersubject variability was not significant ($=$ .05).

TABLE V

SUMMARY ANALYSIS OF VARIANCE TABLE FOR MAXIMAL OXYGEN UPTAKE VALUES

Source	SS	df	MS	F
Between Ss	.733	7	.105	1.27
Between Tests	.699	3	.233	4.18*
Residual	1.060	19	.0557	
Total	2.492	29		

*F.95 (3,19) = 3.13
F.99 (3,19) = 5.01
F.95 (7,22) = 2.46

NOTE: The sum of squares for the between subject and between test variability has been adjusted by the method described in Goulden (23:323) in order to minimize any bias introduced by the estimated values.

A Duncan's New Multiple Range Test was applied to the between test means to determine where the significant differences existed (15:136-140). Table VI summarizes this test. Significant differences were found between the maximal oxygen uptake values of Day 4 and Day 12 as well as between Day 8 and Day 12 ($\alpha = .05$). No other significant differences were found.

TABLE VI

DUNCAN'S NEW MULTIPLE RANGE TEST SUMMARY
ON MAXIMAL OXYGEN UPTAKE VALUES

	1 Day 4	2 Day 8	3 Predonation	4 Day 12	Shortest Significant Ranges
Means	3.643	3.676	3.873	4.025	
3.643	-	.033	.230	.382*	R_2 = .247
3.676		-	.197	.349*	R_3 = .259
3.873			-	.152	R_4 = .267

* Significant at $\alpha = .05$

Trend analyses on the maximal oxygen uptake values showed that the changes over days followed a significant quadratic trend with a F of 12.05 (62:70-71). Critical F values at $\alpha = .05$ and $\alpha = .01$ (degrees of freedom one and 19) were 4.38 and 8.18 respectively. Figure 2 shows the trend of maximal oxygen uptake decreasing from the Predonation level to Day 4 and Day 8, and increasing again to Day 12. Linear and cubic trends (with F's of 1.39 and less than one respectively) were not significant at $\alpha = .05$.

The mean maximal oxygen uptake value for the Predonation test (3.873 $\pm$ .107 liters per minute) reported in this study compare favorably with previous studies, taking into account the subjects' age and normal vigor. Buskirk and Taylor's figure for an 18 to 29 year old group of sedentary students and soldiers was 3.44 $\pm$ 0.46 liters per minute (8). For Mitchell, Sproule, and Chapman's group, aged 20 to 29 years (also sedentary), the corresponding figure was 3.37 $\pm$ 0.51

liters per minute (42). Glassford's values for a more habitually active group of 24 volunteers, students, staff, and army personnel (aged 17 to 33 years) was reported to be 3.758 $\pm$ 0.327 and 3.752 $\pm$ 0.467 liters per minute (22). Figures for active athletes have been shown to be somewhat higher. Astrand's value for 33 Swedish athletes, 20 to 29 years of age was 4.15 $\pm$ 0.36 liters per minute (3) as compared with 4.05 $\pm$ 0.39 liters per minute for 65 young athletes cited by Slonin, Gillespie and Harold (55). Buskirk and Taylor's comparable figure for a group of young trained subjects was 3.95 $\pm$ 0.43 liters per minute (8). It should be noted that the relatively small standard deviation of the present study can most logically be attributed to the subjects' relative homogeneity with regard to habitual activity level.

Trend analyses for maximal oxygen uptake have shown a decreasing tendency from the Predonation test to the Day 4 test. The fact that this difference was found to be insignificant was not entirely unexpected since Balke et al (6) had found that oxygen consumption recovered to the control level in two to three days after the blood donation. The question does arise, however, as to the possible maximal oxygen consumption values during the four days after giving blood. The individuals' capacity for heavy prolonged physical work according to Astrand (3), will at first be dependent upon the supply of oxygen to the working muscles. In physical work, such as the kind used in this study, which engages large groups of muscles the limiting factor for maximal oxygen uptake will probably be the capacity and regulation of the oxygen transporting system (3). Therefore the alteration of the oxygen transporting systems will probably alter the ability of the individual to perform hard physical work. Logically, the depletion of

blood volume itself must affect the dynamics of the cardiovascular system and the concomitant reduction in circulating hemoglobin may impose limitations on blood gas transport. Balke et al (6) reported this one hour after the blood donation. Since the test used by Balke et al (6) was a submaximal test, it is expected that the limitations imposed upon the blood gas transport system would be detectable for a shorter time than would be expected from a maximal test. This is borne out in the observation that the difference between the Predonation test and the Day 4 test was .230 liters of oxygen compared with .247 liters of oxygen required for significance. This would suggest that slower recovery has taken place than that observed by Balke et al (6). Some degree of regeneration and recovery has undoubtedly taken place by Day 4 (6, 25, 47, 56). Day 8 and Day 12 values also not significantly different from the Predonation level (at $\alpha = .05$), would of course, be closer to full recovery than Day 4. However, since Day 12 was the last day examined it is not possible to state whether maximal oxygen uptake would exceed the Predonation level as was found by Balke et al (6), who reported a significant increase over the control level by the eighth day. Howell and Coupe (27) reported trends similar to those of Balke et al (6). Because their control group showed the same trends as their experimental group, they suggested that there must be some degree of emotional contamination of the findings. Dennison also reported similar findings.

The present study employing a maximal test may also account for the apparent stretching out of the trends over a larger period than the submaximal tests of Balke et al (6). Howell and Coupe (27), and Dennison (13). This view may also be supported by Karpovich and

Millman (31), who reported that endurance activities, that depend primarily upon oxygen debt, were affected for a greater time than sprint or submaximal activities.

The significant difference between Day 4 and Day 12 as well as between Day 8 and Day 12 would appear to support the possibility of a significant difference between the Predonation test and a potential test before Day 4.

Consideration of the group as a whole may be misleading. Although the net interindividual difference has been shown to be insignificant by the analysis, an increasing standard deviation over trials would appear to support the observation that individual subjects were recovering at different rates. (Other studies have shown a wide range of interindividual differences with regard to recovery from a blood donation (20, 29, 39, 47).) Considering the subjects as individuals, five of the eight subjects showed an increase over their control level on Day 12. The other three subjects had not as yet reached their control level by the fourth test. It is possible that these subjects might have also exceeded their control effort if examined after an adequate time had elapsed after the blood donation. However, this slower approach may also prevent surpassing the control since homeostatic mechanisms would have had a greater time to achieve normal equilibrium.

Several factors could be responsible for the observed interindividual differences. It has been demonstrated that the administration of iron supplement to subjects after a blood loss speeds up the regeneration of the blood (20). If for any reason an iron deficiency exists in the diet of a donor, his blood will take longer to regenerate. Previous demand upon the erythropoietic system has been shown to govern

the ability of the system to respond to a blood loss (2, 25). Stimulation of the erythropoetic system increases the speed at which it is able to regenerate the loss. The donor's general health at the time of donation and during recovery could also affect speed of regeneration. All other factors being equal, the individual's heredity will govern his ability to recover.

According to the comparative appraisal of the maximal oxygen uptake values, between the group of subjects in this study and previous studies, it is evident that the subjects in this study were above average in fitness as well as being relatively homogenious. Less fit individuals may be affected differently since the fit individuals may be hindered by a ceiling effect on their maximal oxygen uptakes. That is, the less fit individuals may be able to increase their aerobic capacity to a greater extent than the fit individuals since increased blood volume which has been reported (6, 56) may contribute to increasing the capilliary bed. With fit individuals this has already been done to a certain extent.

In fact, if some benefit from the blood donation does exist it is most likely to be transitory unless accompanied by some maintenance procedure. This may take the form of an increased trainability during this period. Instead of the increased volume returning to normal it could then readjust its constituency to the new volume.

Maximal Oxygen Pulse

Mean maximal oxygen pulse values before and at the three specified periods after the blood donation are graphed in Figure 3.

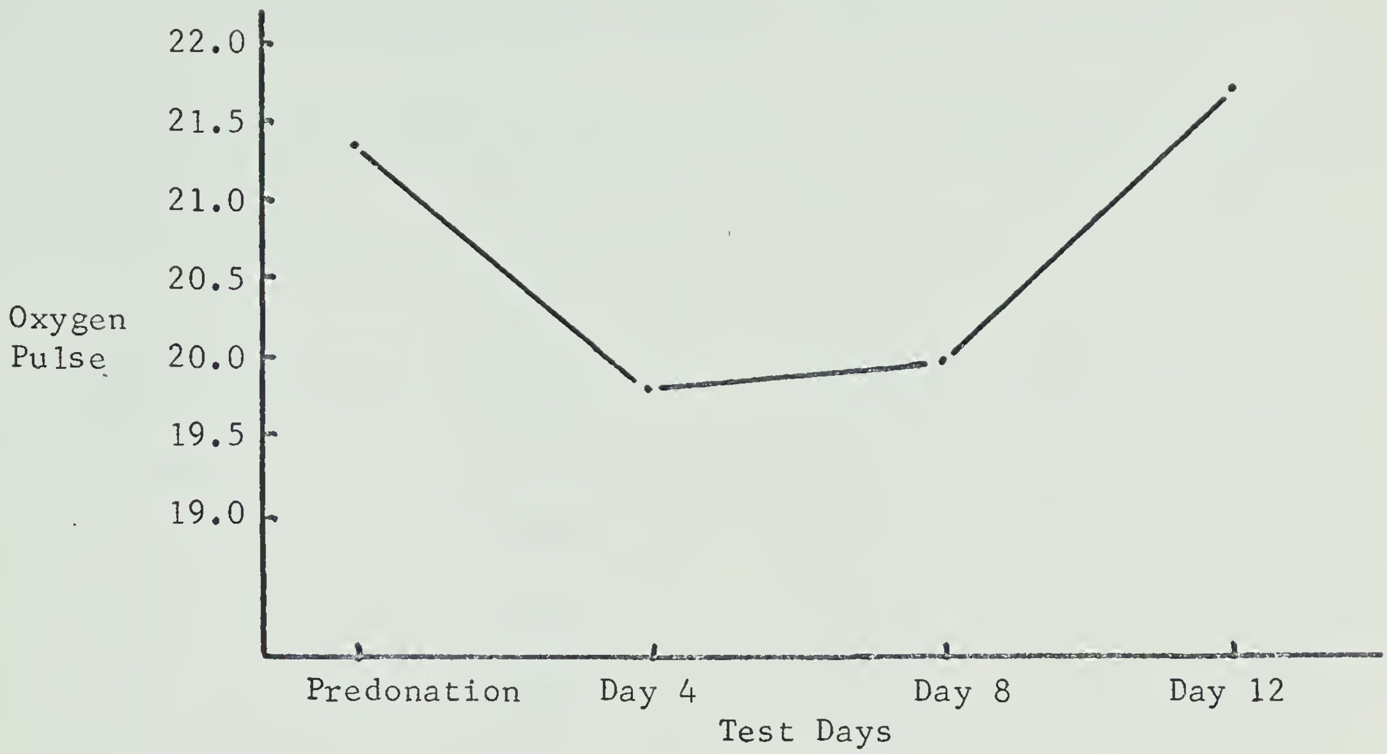

Figure 3: Mean maximal oxygen pulse values (ml O_2 per beat) for the experimental subjects before and at the three specified periods after blood donation.

The obtained values for maximal oxygen pulse, with their means and standard deviations are presented in Table VII. Recall that the values for subjects five and eight (contained in parentheses) have been estimated since they were unavailable. The method of estimation is described by Goulden (23:318-327).

TABLE VII

MAXIMAL OXYGEN PULSE VALUES IN MILLILITERS PER BEAT

Subject	Predonation	Day 4	Day 8	Day 12
1	23.293	21.361	20.608	21.152
2	21.296	17.645	18.113	18.790
3	20.608	19.727	18.535	23.455
4	21.086	21.215	20.073	22.471
5	21.757	18.528	21.068	(21.806)
6	20.396	19.994	17.588	19.694
7	21.727	19.483	23.960	24.866
8	20.941	20.358	(20.036)	21.739
Mean	21.388	19.789	19.998	21.747
S.D.	.849	1.182	1.895	1.820

NOTE: Values in parenthesis have been estimated according to Goulden (23:318-327).

The Cochran Test was used to test for homogeneity of variance between test days (62:94). The obtained C value (.398) was found to be less than the critical C value (.536 at $\alpha = .05$, degrees of freedom four and seven) indicating that the variances were homogeneous to the extent of satisfying that assumption inherent in the analysis of variance.

Table VIII shows the summary analysis of variance for maximal oxygen pulse values presented in Table VII (62:105-132), after appropriate adjustments to the degrees of freedom and sums of squares for

the between subject variation and the between test variation were made. The obtained F ratio (3.75) for the between test variability was significant at the .05 level. Intersubject variability was not significant ($\alpha = .05$).

TABLE VIII

SUMMARY ANALYSIS OF VARIANCE TABLE FOR MAXIMAL OXYGEN PULSE VALUES

Source	SS	df	MS	F
Between Ss	36.142	7	5.163	1.93
Between Tests	21.317	3	7.106	3.75*
Residual	35.988	19	1.894	
Total	93.447	29		

*$F_{.95}$ (3,19) = 3.13
$F_{.99}$ (3,19) = 5.01
$F_{.95}$ (7,22) = 2.46

NOTE: The sum of squares for the between subject and between test variation has been adjusted (23:323).

Table IX summarizes the results obtained when a Duncan's New Multiple Range Test was applied to the between test means to determine between which means significant differences existed (15:136-140). Significant differences were found between the maximal oxygen pulse values of the Predonation day and Day 4 as well as between Day 12 and each of Day 4 and Day 8. No other significant differences were found.

TABLE IX

DUNCAN'S NEW MULTIPLE RANGE TEST SUMMARY ON MAXIMAL OXYGEN PULSE VALUES

	1 Day 4	2 Day 8	3 Predonation	4 Day 12	Shortest Significant Ranges
Means	19.789	19.998	21.388	21.747	
19.789	-	.209	1.599*	1.958*	$R_2 = 1.441$
19.998		-	1.390	1.749*	$R_3 = 1.514$
21.388			-	.359	$R_4 = 1.558$

* Significant at $\alpha = .05$

Trend analyses on the maximal oxygen pulse values (62:70-77) showed that the changes over days followed a significant quadratic trend ($F = 11.83$). Critical F values at $\alpha = .05$ and $\alpha = .01$ (degrees of freedom one and 19) were 4.38 and 8.18 respectively. Figure 3 shows the trend of maximal oxygen pulse decreasing from the Predonation level to Day 4 and Day 8 and increasing to Day 12. Linear and cubic trends (both with F's of less than one) were found to be non-significant at $\alpha = .05$.

In the absence of a direct measurement of cardiac output, an attempt has been made to estimate cardiovascular efficiency on a comparative basis by computing maximal oxygen pulse. Oxygen pulse has been described as an index of the efficiency of cardiovascular adjustment to the increased demands of exertion (48).

In this study maximal oxygen pulse values were found to follow a similar trend to that reported for maximal oxygen uptake values. The significant decrease from the Predonation test to the Day 4 test indicates that less oxygen per pulse was being utilized at the maximal work load level of Day 4 over the Predonation level. The Day 8 maximal oxygen pulse volume indicated no change over Day 4. However, the Day 12 value recovered to the Predonation level. In terms of efficiency of cardiovascular adjustment, efficiency decreased from the Predonation test to the Day 4 test and remained unchanged to the Day 8 test after which it again increased to the Day 12 test. There was no significant difference detected between the oxygen pulse of the Predonation test and the Day 12 test.

Similar trends in maximal oxygen pulse was reported by Balke et al (6), that is, a decrease in oxygen pulse one hour after the donation, followed by an increase to the control level two to three days later, and a further increase above the control level eight to ten days after the donation. Since statistical analysis was not performed on the maximal oxygen pulse values of Balke et al (6) the significance of their reported differences remains questionable.

As in the above case of the maximal oxygen uptake data, consideration of the group of subjects as a whole could be misleading. Similarly to that reported for maximal oxygen uptake, (although the Day 12 values of maximal oxygen pulse were not significantly different from the Predonation values), consideration of the subjects as individuals may help to clarify the results. Five of the eight subjects exceeded their control level on maximal pulse on Day 12. The remaining

three subjects had not yet regained their control level on Day 12. This further supports the claim of interindividual differences with regard to recovery from the effects of a blood donation. Perhaps, if the three subjects were tested after a greater lapse of time following their blood donation, they also may have exceeded their control levels. However, this slow approach to the normal condition may prevent exceeding it as was suggested for maximal oxygen uptake.

In the absence of any significant changes in maximal heart rate (Table XI) the observed changes in maximal oxygen pulse values may be accounted for by three major factors. These are stroke volume, arteriovenous oxygen difference, and the oxygen carrying capacity of the blood. No direct measure was taken on any of these factors, therefore it is not possible to state the role played by each in the observed oxygen pulse changes. However, conjecture may provide a basis for further study.

Kjellberg, Rudhe, and Sjostrand (33) reported a correlation of .90 between blood volume and heart volume. They further reported that "this indicates that there is a close correlation between the total blood volume and the stroke volume during work" (33:49). It is therefore proposed that the depleted blood volume attributed to the blood donation would decrease the stroke volume during work. Stroke volume would then return to normal as the blood volume returns to normal. Overcompensation of blood volume could even increase stroke volume above normal levels (6, 56).

Any possible change in arteriovenous oxygen difference may be more involved. Carbonic acid is the main acid formed in the body from

muscular work (2). It continuously enters tissue capilliories where it is buffered primarily by the potassium salts of hemoglobin inside the blood cells (2). The depletion of blood volume as a result of the donation will deplete red cell volume and buffering agents. In effect, the blood donation may effectively deplete the alkaline reserve permitting a decrease in pH and an increase in carbonic dioxide tension. This would have the effect of shifting the oxygen dissociation curve to the right, thereby limiting carbon dioxide clearing and oxygen uptake. As blood volume returns to normal over the recovery period it would be expected that arteriovenous oxygen difference would return to normal. Any overcompensation of blood volume at the end of the recovery period, if it occurs, could have a beneficial effect upon the arteriovenous oxygen difference by providing a greater alkaline reserve than normally exists.

Changes in the oxygen carrying capacity of the blood may also help to account for the observed maximal oxygen pulse changes. Soon after the blood donation, the plasma volume is expanded by the inflow of protein poor fluid from the interstitial spaces (1, 12, 14). This has the effect of diluting the blood so that the amount of hemoglobin per unit volume is decreased. Even with an increase in stroke volume which might occur as the blood volume increases, maximal oxygen pulse could not increase because of the decreasing ability of the blood to carry oxygen per unit volume. As the hemodilution becomes complete, the hemoglobin per unit volume may increase with the erythropoiesis and in so doing increase the blood's oxygen carrying capacity per unit volume. As the hemoglobin per unit volume reaches normal values total blood volume may well be above normal values. This would permit a

greater stroke volume (as referred to above) and consequently a greater than normal maximal oxygen pulse.

Maximal Heart Rate

The obtained values for maximal heart rate (that is, heart rate concurrent with maximal oxygen consumption), with their means (graphically presented in Figure 4) and standard deviations are found in Table X. Again values for subjects five and eight which were unavailable have been estimated using the method described by Goulden (23: 318-327) and indicated by parentheses.

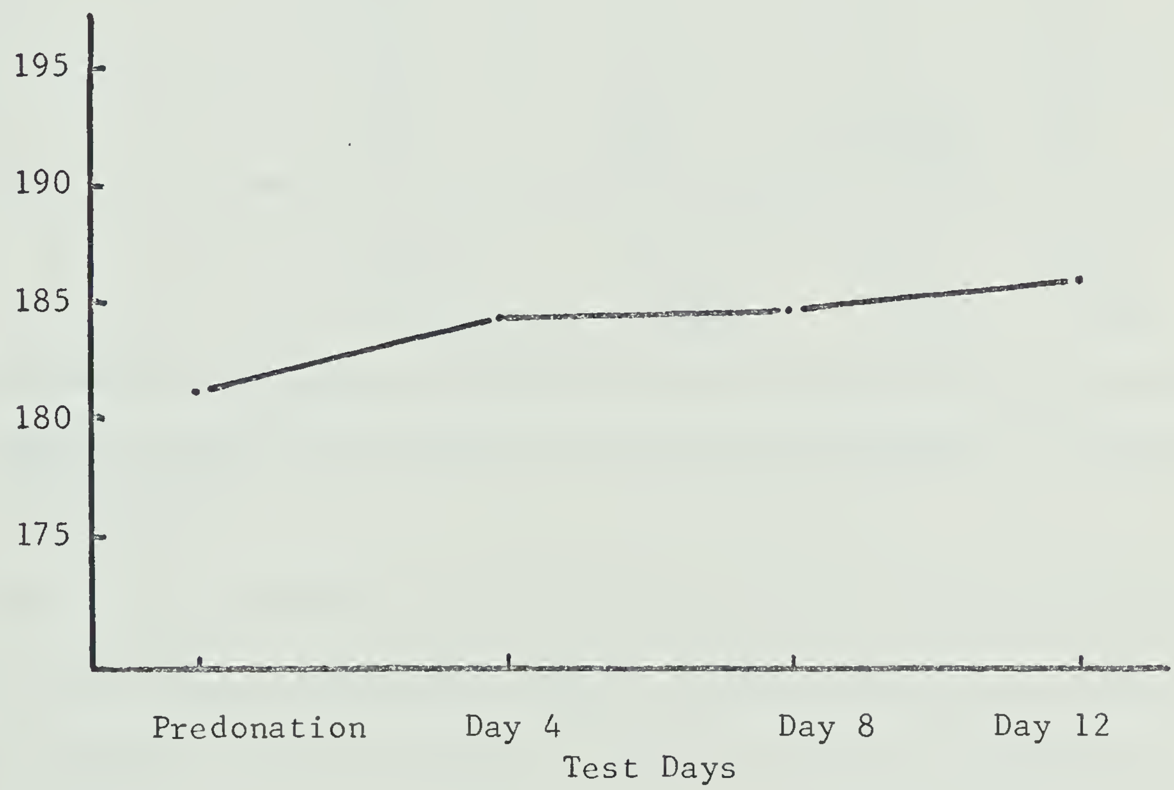

Figure 4: Mean maximal heart rate values (beats per minute) for the experimental subjects before and at the three specified periods after blood donation.

TABLE X

MAXIMAL HEART RATE VALUES IN BEATS PER MINUTE

Subject	Predonation	Day 4	Day 8	Day 12
1	174	180	176	184
2	186	197	195	195
3	181	187	187	191
4	187	186	191	191
5	173	180	176	(178.49)
6	187	177	187	180
7	175	180	176	180
8	187	187	(186.69)	184
Mean	181.25	184.25	184.33	185.54
S.D.	6.34	6.41	7.43	6.15

NOTE: Values in parentheses have been estimated according to Goulden (23:318-327).

The C value obtained (.317) using the Cochran Test for homogeneity of variances between test days (62:94) was found to be less than the critical C value (.536 at $\alpha = .05$ and degrees of freedom four and seven) indicating that the variances were homogeneous, therefore satisfying the assumption inherent in the analysis of variance.

An analysis of variance was performed on the data of Table X (62:105-132) making appropriate adjustments to the degrees of freedom and sums of squares for the between subject variation and the between test variation (23:323). The summary analysis of variance table

(Table XI) shows the obtained F ratio (1.39) for the between test variability was insignificant at $\alpha = .05$. Intersubject variability was found to be significant at $\alpha = .01$ with an F of 8.39.

TABLE XI

SUMMARY ANALYSIS OF VARIANCE TABLE FOR MAXIMAL HEART RATE

Source	SS	df	MS	F
Between Ss	896.05	7	128.01	8.39**
Between Tests	56.84	3	18.95	1.39
Residual	258.16	19	13.59	

$F_{.95}\ (3,19) = 3.13$
$^{**}F_{.99}\ (3,19) = 5.01$
$F_{.95}\ (7,22) = 2.46$

NOTE: The sum of squares for the between subject and between test variability has been adjusted (23:323).

Trend analyses on the maximal heart rates (62:70-77) showed that the changes over days followed a significant linear trend ($F = 4.60$). Critical F values at $\alpha = .05$ (degrees of freedom one and 19) was 4.38. Figure 4 shows the trend of maximal heart rates. Quadratic and cubic trends (with F's of less than one) were found to be non-significant at $\alpha = .05$.

The maximal heart rates in the present study are comparable to those found by other investigators. Coyne (10) reported a mean maximal heart rate of 178.6 ± 5.2 beats per minute for a group of thirty volunteer staff and students. The maximal values for a group of 24 first year physical education students were 182.5 ± 7.8 and

185.2 $\pm$ 8.5 beats per minute (38). Mitchell, Sproule, and Chapman's values for a group of 'normal males' was reported to be 187 $\pm$ 10 beats per minute (42). King, however, reported a higher mean value for 20 physical education majors at 192.5 $\pm$ 7.8 beats per minute (32).

The between subject variation in this study was, as might be expected, significant since the subjects have been shown to recover at different rates from the effects of the blood donation. Heart rate would then, be a reflection of the various circulatory conditions existing throughout the test period. Since no significant differences were found between maximal heart rates over days, the observed changes in maximal oxygen uptake may be attributed to stroke volume, arterio-venous oxygen difference and/or the oxygen carrying capacity of the blood. Changes in maximal heart rates after blood loss, where these changes have been found, last only a short time (less than 24 hours) after the donation (6, 26, 56). Howell and Coupe (27) reported no change in exercise heart rates over seven days after venesection. Balke *et al* (6), however, reported a relative bradycardia accompanying their reported increase in performance eight to ten days after the donation. The comparability of the subjects in this study with those used by Balke *et al* (6) is questionable with regard to maximal heart rate since the subjects in this study have been shown to possess relatively low maximal heart rates. Perhaps an investigation of less fit individuals would give a different picture of maximal heart rates after a blood loss.

Recovery Heart Rate

Recovery heart rate values for five minutes following the subjects' maximal oxygen uptake work load level may be found in Appendix E.

An analysis of variance was performed on the recovery heart rate data for six subjects (62:289). (Data for subjects five and eight were omitted from the analysis, since each contained missing values.) The analysis showed that recovery for minutes (F = 307.70) and for the interaction days by minutes (F = 2.16) were significant at $\alpha = .01$ and $\alpha = .05$ respectively; however the F for days (1.83) was not significant (Table XII).

TABLE XII

SUMMARY ANALYSIS OF VARIANCE TABLE FOR RECOVERY HEART RATES

Source	SS	df	MS	F
Subjects	4997.14	5	999.43	
Days	993.73	3	331.24	1.85
Minutes	97693.31	5	19538.66	307.70**
Subjects x Min.	1587.61	25	63.50	
Subjects x Days	2687.02	15	179.13	
Days x Minutes	1130.85	15	75.39	2.16*
Subj. x Da. x M.	2612.90	75	34.84	
Total	111702.56	143		

$F_{.95}\ (3,15) = 3.29$
$^{*}F_{.95}\ (5,25) = 2.61$
$^{*}F_{.95}\ (15,75) = 1.80$
$^{**}F_{.99}\ (5,25) = 3.86$
$^{**}F_{.99}\ (15,75) = 2.30$

NOTE: The analysis has been done with the omission of subjects five and eight since each had missing values.

Trend analysis on the day by minute interaction (16:314-316) showed significant differences between the linear trends of minutes over days with the trend for the Predonation day being significantly different from each of Day 4, Day 8, and Day 12. There were no significant linear trend differences between Day 4, Day 8 or Day 12. Similarly, significant differences between quadratic trends were found between the Predonation day, and both Day 8 and Day 12. Deviations from linear and quadratic trend differences were not significant. Profiles of these trends for the days by minute interaction are presented in Figure 5.

As would be expected the significant F for minutes is a reflection of the nature of recovery, therefore not warranting further discussion. The primary concern in examining the recovery heart rates was to determine whether the subjects' ability to recover from the exercise (as reflected by heart rate) was affected by the loss of blood or its subsequent regeneration. Since the maximal heart rates over days were not significantly different (Table XI) recovery has taken place from essentially the same level and therefore would be comparable. The finding that the Predonation recovery heart rate was slower than any of the subsequent recovery heart rates with respect to the linear component and also slower than the recovery heart rates of Day 8 and Day 12 with respect to the quadratic component is difficult to support unless it could be assumed that the blood donation had some beneficial effect upon recovery heart rate. However, it would seem more likely and is suggested that these differences may be due to psychological factors rather than factors inherent in or resulting from the blood donation. Since recovery from the maximal level is a return to submaximal physiological conditions where emotion could affect

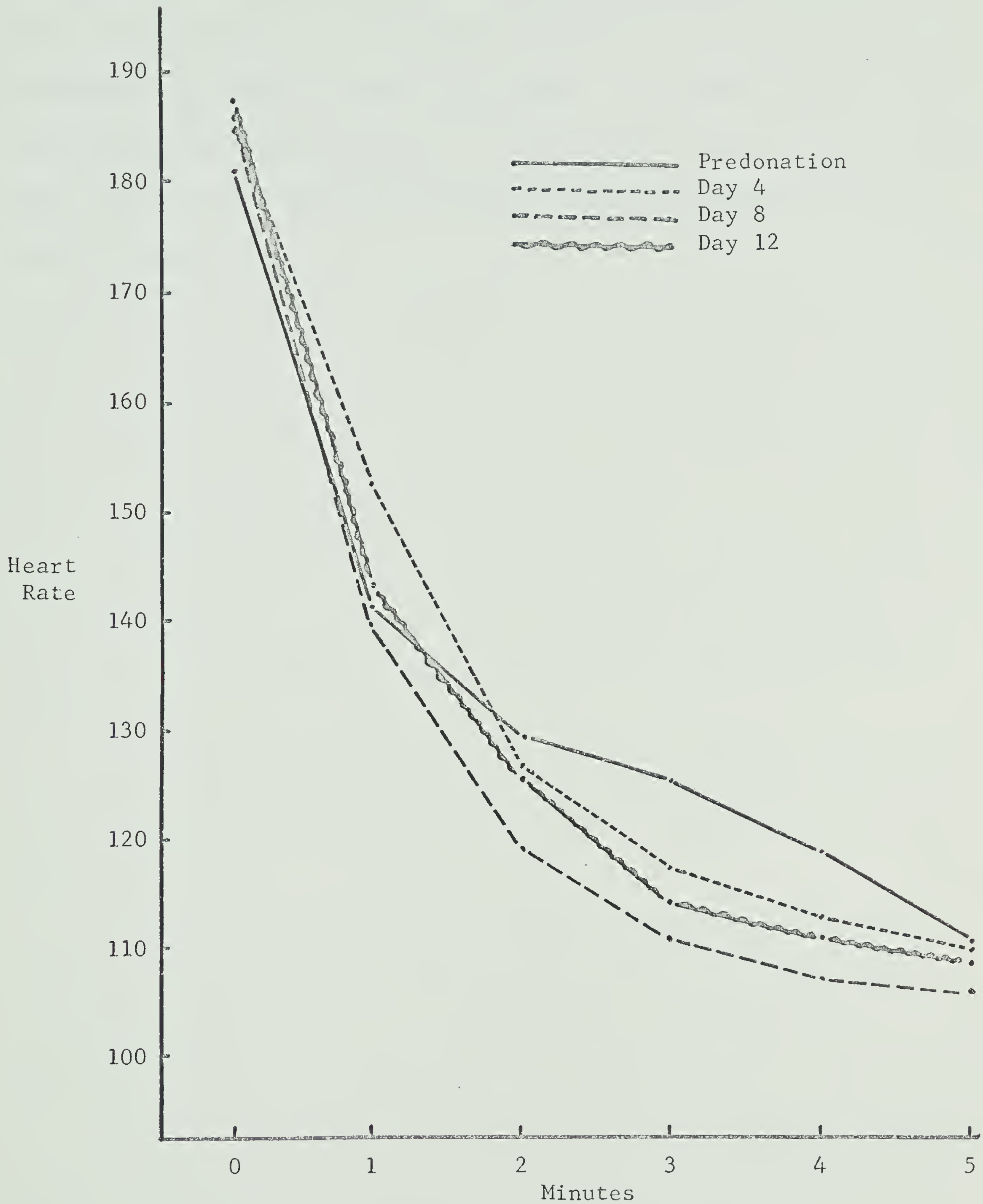

Figure 5: Recovery heart rates (beats per minute) for five minutes following the maximal work load level.

the results, The first test recovery heart rate pattern could be the result of the subjects' apprehension toward the test as a whole and in particular the next work load level, since it was each subject's first encounter with an exhaustion test. In subsequent tests, recovery heart rates could be a reflection of boredom replacing emotion at the submaximal levels (58).

CHAPTER V

SUMMARY AND CONCLUSIONS

It was the purpose of this study to examine the effect of a standard blood donation of 500 cc on the physiological parameters of maximal oxygen uptake, maximal oxygen pulse, and maximal heart rate at intervals of four days, eight days, and twelve days after venesection. A subsidiary examination was also made on recovery heart rate for five minutes following the maximal work load level. These physiological parameters were elicited by a modified version of the maximal oxygen consumption test described by Mitchell, Sproule and Chapman (42).

The experimental group consisted of eight healthy male subjects attending the 1967-68 session at the University of Alberta. All were paid volunteers.

After an initial orientation period the subjects took a Pre-donation test for control purposes within one week of their blood donation. Subsequent tests were taken at intervals of four days, eight days, and twelve days after the blood donation.

The test data was analyzed by an analysis of variance technique and the Duncan's New Multiple Range test. Trend analysis were incorporated to examine the changes over days and to compare recovery heart rates between days. Significance was accepted at the 95 per cent level of confidence.

Conclusions

Within the limitations of the study and the reliability of the experimental and statistical procedures, the following conclusions appear to be warranted.

1. By the fourth day after blood donation, performance on the maximal oxygen uptake test did not differ from the control level or the levels on subsequent test days.
2. Maximal oxygen pulse was significantly less on the fourth day after blood donation indicating that efficiency of cardio-vascular adjustment to the stress of exertion had decreased over the control level.
3. Interindividual differences in recovery from the effects of blood loss on maximal oxygen uptake and maximal oxygen pulse were considerable as reflected by increasing standard deviations over days and individual consideration.
4. Maximal heart rate was not significantly affected by the blood loss over the days examined.
5. Recovery heart rate was found to be significantly slower on the Predonation day than on any other day tested.

Recommendations

1. A similar study should be designed using non-athletic subjects to determine if the effects of blood donation are pronounced more than with athletic subjects. A random sample would be most desirable.
2. An effort should be made to examine further the possibility of a beneficial effect of blood donation as suggested by Balke

et al (6) and the present study. This may be best approached by investigating the period after about the eighth day.

3. Measures of stroke volume and arterio-venous oxygen difference would help to clarify the nature of the effect of blood donations on cardiorespiratory function. Measures of hemoglobin concentration, blood volume, reticulocytes, etc. would give an index of blood regeneration paralleling observed effects of the donation.

4. The possibility of a period of increased trainability after a blood donation should be investigated.

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APPENDICES

APPENDIX A

INSTRUCTIONS AND INFORMATION

INSTRUCTIONS AND INFORMATION

(For Subjects Participating in an Experiment for W.C. Brown)

The object of this experiment is to examine the effects of a blood donation on the subject's oxygen consumption and heart rate before and at three specified intervals after blood is given. Because of the nature of the experiment and certain other limitations it has been necessary to select only eight subjects. Therefore it will be essential that each subject make every effort to perform each test. Subjects will be paid a total of eight dollars apiece for completing the tests.

Four tests plus a blood donation at the Red Cross Blood Donation Clinic in the Students' Union Building will be required of each subject.

Test 1 - to be taken prior to donating blood. As far as possible the time will be arranged at the subject's convenience.

Blood Donation - one pint of blood donated sometime before Test I.

Blood Clinic Hours - Monday, Oct. 30 to Friday, Nov. 3
- Monday, Nov. 6 to Thursday, Nov. 9
10:00 a.m. to noon
and 1:30 to 5:30 p.m.

Test 2 - to be taken four days after the donation.
Test 3 - to be taken eight days after the donation.
Test 4 - to be taken twelve days after the donation.

Below is a list of all possible dates for blood donation and post donation tests. Subjects may select any convenient sequence (as far as possible) for testing.

Test 1	Blood Donation	Test 2	Test 3	Test 4
Anytime before blood donation	Mon., Oct. 30	Fri., Nov. 3	Tue., Nov. 7	Sat., Nov. 11
	Tues., Oct. 31	Sat., Nov. 4	Wed., Nov. 8	Sun., Nov. 12
	Wed., Nov. 1	Sat., Nov. 5	Thur., Nov. 9	Mon., Nov. 13*
	Thur., Nov. 2	Mon., Nov. 6	Fri., Nov. 10	Tue., Nov. 14
	Fri., Nov. 3	Tue., Nov. 7	Sat., Nov. 11	Wed., Nov. 15
	Mon., Nov. 6	Fri., Nov. 10	Tue., Nov. 14	Sat., Nov. 18
	Tue., Nov. 7	Sat., Nov. 11	Wed., Nov. 15	Sun., Nov. 19
	Wed., Nov. 8	Sun., Nov. 12	Thur., Nov. 16	Mon., Nov. 20
	Thur., Nov. 9	Mon., Nov. 13*	Fri., Nov. 17	Tue., Nov. 21

* University Holiday - testing as usual

Previous investigators have shown that several conditions may affect the results of the test to be given in this experiment, therefore:

Subjects are requested to observe the following procedures -

1. No smoking within 2 hours prior to the test.

2. No abnormal or strenuous exercise prior to testing.

3. No meals should be eaten within $1\frac{1}{2}$ hours prior to testing.

4. Gym shoes, T-shirt and shorts should be worn for the testing.

Your cooperation and participation is greatly appreciated. Should any subject wish to contact me, my phone number is 433-6534.

W. C. Brown

APPENDIX B

GAS ANALYSIS COMPUTATION

GAS ANALYSIS COMPUTATION

Subject no. ____________

Test no. ______________

Name ______________________________

Date ______________________________

Temperature _______________________

Barometric Pressure _______________ mm. Hg.

Treadmill Inclination _____________ %

$\% O_{2E}$ = _______________ X 2.5 = ________________

$\% N_{2E}$ = 100 - ____________ $\% O_{2E}$ - _______________ $\% CO_{2E}$ = __________

V_EATPS (corrected) = _________ 1. (.982 - .345) = _________________ 1.

V_ESTPD = ______________(Factor) X __________ 1./min.= __________ 1./m.

V_ISTPD = ______________ V_ESTPD X $\frac{\_\_\_\_\_\_\_\_\_\_}{79.04}$ $\% N_{2E}$ = ____________ 1./m.

VO_2 consumption = (____________ V_ISTPD X .2093)

- (____________ V_ESTPD X . _____________ O_{2E})

= ______________ 1./min.

Heartrate

Exercise _____________

Rest 1. _____________

2. _____________

3. _____________

4. _____________

5. _____________

APPENDIX C

APPARATUS CALIBRATION

APPARATUS CALIBRATION

Volume Meters

Accuracy of the American Meter Company gasometer (model 802) was determined by evacuating known quantities of gas from a Collins 120 liter chain compensated gasometer. Errors were corrected by means of the following regression equation.

$$y = .982x - .345$$

x refers to the volume indicated by the American volume meter and y represents the corrected volume at atmospheric temperature and pressure saturated.

Treadmill

The elevation scale of the treadmill was checked by means of a spirit level with an appropriate elevation scale.

Electrocardiograph

The Sanborn 100 Viso-Electrocardiogram chart speed was checked by means of a stop watch.

Gas Analyses

Beckman #E-2 Oxygen Analyzer. The oxygen analyzer scale was set using reference gases containing no oxygen and containing atmospheric oxygen (room air) respectively.

Godart #KK Capnograph. The carbon dioxide analyzer scale was set using gases containing no carbon dioxide and 3.29% carbon dioxide respectively. The percentage carbon dioxide was checked by both the scholander technique and a gas chromatograph.

APPENDIX D

SUBJECT DATA

TABLE XIII

SUBJECT DATA

Subject	Age (years)	Height (inches)	Weight (pounds)	Smoke	Previous Donations
1	27.8	72	175	No	Yes
2	18.5	69	154	No	No
3	19.0	70	158	No	No
4	18.8	72	181	Yes	Yes
5	25.7	72	165	No	Yes
6	18.3	68.5	158	No	No
7	24.7	71.5	183	No	Yes
8	24.8	70	185	No	Yes

APPENDIX E

RAW DATA

TABLE XIV

EXHAUSTION TEST AND MAXIMAL HEART RATE RAW DATA*

Subject	Test	Measure	Per cent Treadmill Inclination 7½	10	12½	15	17½
1	Pre	$\%CO_2$	3.65	2.95	2.90		
		$\%O_2$	17.130	17.180	17.500		
		VE	90.161	102.091	114.775		
		VO_2	3.469	4.053	4.107		
		HR	164	174	170		
	Day 4	$\%CO_2$	3.75	3.55	3.15	2.45	
		$\%O_2$	17.445	17.445	17.600	17.575	
		VE	86.220	93.903	113.556	101.562	
		VO_2	2.957	3.270	3.845	3.655	
		HR	173	176	180	180	
	Day 8	$\%CO_2$	3.50	3.35	3.10		
		$\%O_2$	17.315	17.372	17.740		
		VE	86.528	100.090	113.996		
		VO_2	3.158	3.627	3.673		
		HR	170	176	180		
	Day 12	$\%CO_2$	3.75	3.45	3.15	3.00	
		$\%O_2$	16.925	17.225	17.675	17.315	
		VE	86.707	100.461	118.490	101.515	
		VO_2	3.544	3.804	3.892	3.839	
		HR	173	170	184	181	

2	Pre	$\%CO_2$	4.00	3.75	3.65	
		$\%O_2$	16.597	16.948	17.488	
		VE	82.689	97.831	91.692	
		VO_2	3.659	3.961	3.113	
		HR	168	186	184	
	Day 4	$\%CO_2$	4.00	3.90	3.70	3.50
		$\%O_2$	16.325	16.705	16.844	17.334
		VE	64.823	74.710	82.734	84.003
		VO_2	3.098	3.231	3.476	3.053
		HR	180	191	197	198
	Day 8	$\%CO_2$	3.50	3.50	3.50	3.45
		$\%O_2$	16.925	17.025	17.50	17.405
		VE	71.031	82.067	88.555	98.083
		VO_2	2.950	3.304	3.532	3.491
		HR	167	191	195	195
	Day 12	$\%CO_2$	3.75	4.20	4.20	4.15
		$\%O_2$	17.205	16.375	16.512	16.850
		VE	60.777	73.647	81.689	90.886
		VO_2	2.267	3.425	3.664	3.707
		HR	167	191	195	200

3	Pre	$\%CO_2$	3.90	3.65	3.25	3.25	
		$\%O_2$	16.685	17.000	17.372	17.662	
		VE	78.331	87.201	102.201	106.639	
		VO_2	3.408	3.499	3.730	3.529	
		HR	183	184	181	191	
	Day 4	$\%CO_2$	3.65	3.70	3.60	3.45	
		$\%O_2$	17.020	16.675	16.900	17.381	
		VE	70.212	72.539	88.864	105.476	
		VO_2	2.799	3.194	3.689	3.781	
		HR	173	184	187	191	
	Day 8	$\%CO_2$	3.60	3.50	3.30	3.20	
		$\%O_2$	16.850	16.738	17.137	17.137	
		VE	71.492	75.950	88.253	87.440	
		VO_2	3.013	3.327	3.466	3.458	
		HR	161	184	187	187	
	Day 12	$\%CO_2$	4.00	3.75	3.65	3.05	2.90
		$\%O_2$	15.800	16.250	16.275	16.705	16.575
		VE	66.753	81.945	86.899	98.444	87.055
		VO_2	3.630	4.043	4.278	4.480	4.128
		HR	170	180	191	191	191

4	Pre	$\%CO_2$	4.15	3.50	2.40		
		$\%O_2$	17.550	16.970	17.775		
		VE	83.930	96.411	98.308		
		VO_2	2.672	<u>3.943</u>	3.300		
		HR	184	187	191		
	Day 4	$\%CO_2$	4.10	3.70	3.25		
		$\%O_2$	16.525	16.882	17.525		
		VE	76.922	95.082	114.906		
		VO_2	3.462	<u>3.946</u>	3.976		
		HR	180	186	200		
	Day 8	$\%CO_2$	4.00	3.60	3.20		
		$\%O_2$	16.650	16.975	17.562		
		VE	82.364	94.642	113.399		
		VO_2	3.593	<u>3.834</u>	3.882		
		HR	176	191	195		
	Day 12	$\%CO_2$	4.35	4.15	3.65	3.20	2.90
		$\%O_2$	16.288	16.505	17.050	17.275	17.623
		VE	76.719	86.789	105.340	113.636	107.388
		VO_2	3.625	3.916	4.159	<u>4.292</u>	3.680
		HR	180	191	200	191	193

5	Pre	$\%CO_2$	3.90	3.90	3.75	3.50	
		$\%O_2$	16.100	16.005	16.380	17.150	
		VE	60.666	67.106	78.913	94.388	
		VO_2	3.084	3.497	3.764	3.645	
		HR	161	173	173	180	
	Day 4	$\%CO_2$	4.10	4.00	3.95	3.60	3.40
		$\%O_2$	16.275	16.400	16.658	17.050	17.318
		VE	61.083	66.618	73.857	84.170	92.222
		VO_2	2.934	3.117	3.222	3.335	3.388
		HR	167	173	180	180	178
	Day 8	$\%CO_2$	4.30	4.20	4.15	3.75	3.40
		$\%O_2$	16.025	16.100	16.375	16.555	17.318
		VE	62.992	69.472	76.796	81.648	87.791
		VO_2	3.200	3.477	3.582	3.708	3.163
		HR	129	173	176	176	181
	Day 12		-				
			-				

6	Pre	$\%CO_2$	4.40	4.40	3.50	3.00
		$\%O_2$	15.525	15.398	16.900	17.530
		VE	54.235	59.028	91.289	105.600
		VO_2	3.084	3.446	3.814	3.711
		HR	161	176	187	188
	Day 4	$\%CO_2$	3.85	3.50	3.40	
		$\%O_2$	16.237	16.850	17.325	
		VE	67.298	83.432	92.995	
		VO_2	3.311	3.539	3.416	
		HR	184	177	187	
	Day 8	$\%CO_2$	3.60	3.10	2.95	2.30
		$\%O_2$	17.000	17.400	17.800	18.150
		VE	72.809	85.132	103.250	111.369
		VO_2	2.931	3.109	3.289	3.247
		HR	167	176	187	187
	Day 12	$\%CO_2$	4.00	3.65	3.45	
		$\%O_2$	16.712	16.88	17.354	
		VE	77.082	86.240	98.906	
		VO_2	3.304	3.545	3.583	
		HR	173	180	176	

7	Pre	$\%CO_2$	4.25	3.75	3.50	
		$\%O_2$	16.670	16.425	17.275	
		VE	74.288	80.557	100.412	
		VO_2	3.173	<u>3.802</u>	3.713	
		HR	164	175	180	
	Day 4	$\%CO_2$	4.35	3.50	3.65	
		$\%O_2$	16.325	16.975	16.808	
		VE	67.704	86.011	72.717	
		VO_2	3.173	<u>3.507</u>	3.092	
		HR	155	180	173	
	Day 8	$\%CO_2$	4.35	4.25	3.90	3.50
		$\%O_2$	16.10	16.425	16.625	16.850
		VE	74.414	90.197	95.143	97.765
		VO_2	3.695	4.137	4.217	4.147
		HR	158	173	176	172
	Day 12	$\%CO_2$	4.65	4.25	4.15	3.75
		$\%O_2$	16.792	15.980	16.388	16.830
		VE	71.996	81.320	96.222	92.511
		VO_2	2.889	4.182	<u>4.476</u>	3.886
		HR	164	161	180	182

8	Pre	$\%CO_2$	3.85	3.70	3.50	3.20
		$\%O_2$	16.590	16.712	17.000	17.450
		VE	71.668	81.981	96.642	109.953
		VO_2	3.209	3.579	<u>3.916</u>	3.917
		HR	173	180	187	191
	Day 4	$\%CO_2$	3.85	3.35	3.20	2.95
		$\%O_2$	16.500	17.188	17.250	17.450
		VE	74.142	93.598	99.782	101.387
		VO_2	3.404	3.605	<u>3.807</u>	3.678
		HR	164	180	187	191
	Day 8		-			
			-			
	Day 12	$\%CO_2$	4.35	4.20	4.00	
		$\%O_2$	16.245	16.150	16.550	
		VE	71.964	80.954	89.176	
		VO_2	3.446	<u>4.000</u>	4.003	
		HR	170	184	184	

* $\%CO_2$ - per cent carbon dioxide expired
$\%O_2$ - per cent oxygen expired
VE - volume of air expired per minute (liters) at S.T.P. dry
VO_2 - liters of oxygen consumed per minute
HR - heart rate per minute

TABLE XV

RECOVERY HEART RATE RAW DATA

Subject	Recovery Minute	Predonation	Day 4	Day 8	Day 12
1	0	174	180	176	184
	1	128	150	129	132
	2	117	127	115	114
	3	105	115	97	103
	4	99	115	98	107
	5	98	113	92	106
2	0	186	197	195	195
	1	136	156	145	145
	2	130	138	118	129
	3	143	134	117	115
	4	125	127	106	118
	5	120	125	102	118
3	0	181	187	187	191
	1	167	150	138	155
	2	141	115	115	127
	3	141	117	108	115
	4	135	111	113	110
	5	123	113	110	92
4	0	187	186	191	191
	1	148	141	138	155
	2	127	129	122	143
	3	123	120	117	129
	4	123	117	108	123
	5	118	114	110	123
5	0	173	180	176	-
	1	115	127	105	-
	2	107	117	105	-
	3	115	107	107	-
	4	106	97	96	-
	5	89	95	92	-
6	0	187	177	187	180
	1	143	150	145	134
	2	130	122	129	127
	3	125	118	122	122
	4	120	119	115	117
	5	118	111	122	118

7	0	175	180	176	180
	1	134	164	141	138
	2	130	123	118	115
	3	121	100	105	108
	4	106	93	100	99
	5	91	86	101	95
8	0	187	187	-	184
	1	141	143	-	129
	2	108	107	-	100
	3	101	101	-	103
	4	105	97	-	100
	5	105	97	-	100